**Rejection of Claims Under 35 U.S.C. §112, first paragraph**

*Enablement:*

The Examiner has rejected claims 1, 6, 7, 10, 11, 15, 20-24, 34, and 41-55 under 35 U.S.C. §112 first paragraph because "the specification, while being enabling for a complex comprising an anti-biotin antibody and biotinylated eosin (sic) and a complex comprising an anti-biotin antibody and biotinylated ITAC, does not reasonably provide enablement for complexes consisting of any other biotinylated conjugate." The Examiner specifically states that "the specification does not teach a use for agonistic or antagonistic complexes comprising any other chemokine or ligand that would bind to a G-protein coupled receptor" and that "one of skill in the art would not know how to use the broadly claimed chemokine or G-protein coupled ligands in biotinylated complexes as the specification does not teach or demonstrate any clinical disease states that could be ameliorated by said complexes." The Examiner also states that, with respect to dual specificity antibodies, undue experimentation would be required "to determine optimum binding affinities for both biotin and the tumor antigens." Finally, the Examiner states that "the specification provides no teachings on specific chemokines which would differentially recruit Th1 or Th2 CD+4 T-cells."

Applicants respectfully traverse the rejection for the reasons set forth below.

The enablement standard for a disclosure is whether it would require undue experimentation to practice the invention by one of ordinary skill in the art. An enabling disclosure teaches an ordinary artisan how to make and use the invention without undue experimentation. Although the experimentation may be complex, it still is not be undue if the art regularly engages in that level of experimentation. Factors to be considered in deciding whether the experimentation is undue include: (1) the breadth of the claims, (2) the nature of the invention, (3) the state of the prior art, (4) the level of one of ordinary skill in that art, (5) the level of predictability in the art, (5) the amount of direction provided by the inventor, (6) the existence of working examples, and (7) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. In re Wands, 858 F.2d 731; 8 USPQ 2d 1400 (Fed. Cir. 1988).

Breadth of the Claims: The rejected claims relate to complexes of biotinylated pharmacologically active chemokines and anti-biotin antibodies. The pharmacologically active chemokine may be an agonist or an antagonist, depending upon the embodiment. The antibodies may be full antibodies or fragments. The affinity of the antibodies may range from 1nM to 100nM and in some instances will range from 0.1nM to 1nM. The antibodies may have dual specificity. Further, the antibodies may be further modified by conjugation to diagnostic or therapeutic agents.

Nature of the Invention: The invention relates in part to the ability to modulate the presence and concentration of pharmacologically active agents, particularly chemokines, using complexes of biotinylated versions of these agents and anti-biotin antibodies. The invention overcomes the need to conjugate each ligand individually with a separate label. Furthermore, the present invention facilitates targeting,administration, and concentration of the complexes to desired cells and tissues for a variety of disorders (see e.g., pp. 17, lines 28-30; page 19, lines 22-23; page 25, lines 25-30).

State of the Prior Art at the Time of Filing: Appendix B includes references that evidence the state of knowledge and the level of skill in the art at or before the time of filing. The individual elements of the claims, including chemokines, biotin molecules, anti-biotin antibodies and fragments thereof, linkers and therapeutic and diagnostic agents were well known in the art at the time of filing.

The actions of a vast majority of chemokines listed in the specification were known at the time of filing. The prior art also taught chemokine antagonists in the form of truncated versions, such as truncated IL-8 and MCP-1. (See e.g., page 14, lines 29-32 and page 15, lines 4-6).

Methods for conjugating biotin molecules to an active agent were known in the art (See references cited in Example 1). Moreover, commercial sources (e.g., R&D Systems) for biotinylated chemokines were available. (See page 35, lines 13-15).

Methods for making anti-biotin antibodies were known in the art at the time of filing, as were anti-biotin antibodies. Methods for conjugating diagnostic or therapeutic agents to antibodies were also known at the time of filing, as were antibodies conjugated to such agents. Methods for forming antibody-antigen complexes were also known at the time of filing.

The Level of One of Ordinary Skill in the Art: The requisite level of skill for performing the invention includes the ability to chemically modify a pharmacologically active agent (e.g., by conjugation to biotin) or an antibody (e.g., by conjugation to diagnostic or therapeutic agents). This level of skill also includes the ability to test the binding affinity and efficacy of various biotinylated active agents, anti-biotin antibodies and agent-antibody complexes in vitro and in vivo. The prior art references of Appendix B are evidence that the ordinary artisan is capable of performing chemical modification and screening assays of the type envisioned by the invention.

The Level of Predictability in the Art: As discussed above, the prior art at the time of filing was replete with references describing the individual elements of the claims. Together with the Examples in the specification, one of ordinary skill in the art would reasonably expect that complexes of biotinylated

active agents and anti-biotin antibodies could be prepared, particularly since the components of these complexes were either known or methods of making them were taught in the art. Moreover, the art would also expect that certain disorders could be treated with the claimed compositions because particular G-protein coupled receptor ligands were previously proposed as therapeutic agents for particular conditions. (See references in Appendix B.) The Examples further indicate that the complexes of the invention are functionally efficacious. Accordingly, making the complexes of the invention is predictable and using the complex to modulate concentration in vivo is also predictable, particularly in view of the Examples.

The Amount of Direction Provided by the Inventor: The disclosure of the specification, combined with the knowledge of one of skill in the art teaches the individual elements to be used in the invention, modifications to such active agents in order to create agonists or antagonists, biotinylation of the active agents including linkers to be used and attachment regions most preferred, production and screening of anti-biotin antibodies, conjugation of diagnostic or therapeutic agents to these antibodies, complex formation between the biotinylated active agents and the anti-biotin antibodies, screening of the biological activity of such complexes, and administration of these complexes (as well as of free biotin) to subjects in need of such treatment.

The specification provides guidance regarding several chemokines, many of which are commercially available (e.g., from suppliers such as Pharmingen and R&D Systems). See page 4, lines 19-31; page 12, lines 21-31; and page 13, lines 1-2, page 1, lines 20-26; and pages 44-45 (Table 1). The specification teaches that truncation or deletion of the highly basic carboxyl terminus of chemokines can create chemokine agonists with improved half-life characteristics. (See page 14, lines 24-26.) The specification further teaches that biotinylated truncated or elongated chemokine peptides particularly at the amino terminus can be used as chemokine antagonists that inhibit the normal pharmacological activity of the chemokine. (See page 14, lines 26-29.) Examples of antagonists include biotinylated truncated chemokines having biotin covalently coupled to the carboxyl terminus of a chemokine that lacks a portion of its amino terminal sequence such as the portion N-terminal to the CXC or CC sequence in the CXC and CC families of chemokines. (See page 15, lines 1-4.)

The anti-biotin antibodies of the invention may be antibodies, dual specificity antibodies, or antibody fragments, with affinity from 1 nM to 100nM and in some instances will range from 0.1 nM to 1 nM. (See page 18, lines 29-32; page 19, lines 1-5.) The specification teaches that antibodies having a significantly shorter half-life than avidin-biotin complexes in the presence of

a supra physiological level of free biotin are appropriate for some embodiments. Preferably the complexes of the invention have a half-life on the order to one day to one month and more preferably one week to about two weeks. The specification further teaches that the anti-biotin antibodies are to be selected on the basis of their ability to dissociate from the biotinylated active agent only in the presence of supra physiological levels of free biotin. (See page 6, lines 2-4.) The Examiner's assertion that antibodies with low affinity for biotin would release from the complex within 15 minutes of injection and thus be unable to target a tumor or a location of infection is unfounded, since the specification explicitly teaches that the dissociation of the antibody from the complex would occur only in the presence of supra physiological levels of free biotin and not at normal physiological levels of biotin (which would exist upon injection). Free biotin is administered to a subject in order to achieve supra physiological levels of free biotin following the administration of the antibody-active agent complex in embodiments where it is desired to dissociate the complex,. The specification teaches that the complexes of the invention should have a half life of 20 days, 2 days, and in some instances 0.2 days (i.e., 4.8 hours) in the presence of normal physiological levels of biotin. (See page 18, lies 5-7.) It is only once a supra physiological level of free biotin is induced in a subject by exogenous administration, that the half-life of the complex decreases to less than an hour, less than a half hour and most preferably less than 15 minutes. (See page 6, lines 25-29 and page 18, lines 8-11.) Moreover, the specification teaches that it may be desirable to use antibodies with a higher affinity (e.g., 1 nM to 0.1 nM) that impart a longer half-life to the complex in some instances (e.g., in the treatment of cancer and chronic inflammatory diseases. (See page 19, lines 20-23.)

The present disclosure further provides uses for such compositions including treatment of disorders such as chronic inflammatory diseases, cancer, infections such as viral infections, disorders associated with a lack of a Th1 immune response (e.g., lupus and arthritis), disorders associated with a lack of a Th2 immune response (e.g., leprosy, asthma, inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, and diseases associated with fibrosis such as emphysema and hepatic fibrosis). (See page 17, lines 28-30; page 19, lines 22-23; page 25, lines 25-30.) The actions of a vast majority of the active agents are listed in the specification and were known at the time of filing. The specification provides numerous examples of active agents for treating particular disorders. For example, if a Th1 response is desired for the treatment of cancer, then IP-10, MIG, RANTES and ITAC are recommended. If a Th2 response is desired for

the treatment of e.g., asthma or leprosy, then eotaxin, TARC and MDC are recommended. Accordingly, determining which active agents are best suited to which conditions is within the skill of the ordinary artisan.

As noted above, the invention is an improvement over existing therapeutic methods by providing a means to easily modulate the presence and concentration of a preselected therapeutic agent. Accordingly, the invention involves using a preselected agent for treating a condition in combination with biotin and an anti-biotin antibody.

Working Example: As the Examiner acknowledges, the specification contains working examples of anti-biotin antibody complexes with biotinylated chemokines (biotinylated ITAC and biotinylated eotaxin). The Examples teach biotinylation of the chemokines, preparation and screening of the anti-biotin antibodies, screening of biotinylated chemokine for binding to a cognate receptor and for chemotactic activity, and biological activity of complexes of biotinylated active agent and anti-biotin antibodies. The Examples further demonstrate that the biotinylated active agents and complexes containing such agents possess functional activity in vitro and in vivo.

Thus, in view of the the breadth of the claims and nature of the invention, in light of the state of the prior art, the level of one of ordinary skill and predictability in the art, and the amount of direction provided by the inventor, including the of working examples of the present application, Applicant respectfully submits that the specification provides sufficient description for enablement of the invention as claimed. Accordingly, based on the invention disclosure combined with the state of the art at the time of filing, Applicants assert that the quantity of experimentation needed to make and use the invention is not undue and, more importantly, is within the degree of experimentation routinely practiced by persons of ordinary skill in the art.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the enablement rejection of claims 1, 6, 7, 10, 11, 15, 20-24, 34 and 41-55 under 35 U.S.C. 112, first paragraph.

*Written Description:*

Claims 1, 6, 7, 10, 11, 15, 20-24, 34 and 41-55 are rejected under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." According to the Examiner, "the written description ... is not commensurate in scope with the claims drawn to compositions comprising a biotinylated

pharmaceutical agent complexed to anti-biotin antibodies." The Examiner states that "the specification is not enabling for the broadly claimed pharmaceutical agents" and that "no disclosure, beyond the mere mentioning of the inclusion of all chemokines and pharmaceutical agents is made in the specification." The Examiner concludes that there is insufficient support for the generic claims and that "only an anti-biotin antibody complexed to biotinylated eotaxin and a (sic) anti-biotin antibody complexed to biotinylated ITAC, but not the full breadth of the claims meets the written description provision." Applicants respectfully traverse the rejection for the reasons set forth below.

The Written Description Examination Guidelines ("the Guidelines") indicate that genus claims meets the written description requirement if a representative number of species is implicitly or explicitly disclosed. According to the Guidelines, "a representative number" of species will depend upon whether one of skill in the art would recognize the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed or claimed.

Claims 1 and 34 relate to the genus of complexes of biotinylated pharmacologically active agents and anti-biotin antibodies. As acknowledged by the Examiner, the specification discloses species including complexes of biotinylated ITAC and anti-biotin antibodies and complexes of biotinylated eotaxin and anti-biotin antibodies. Applicants were in possession of the common attributes or features possessed by the members of the genus at the time the invention was made, as detailed below.

First, the members of the genus commonly possess a pharmacologically active agent in the form of chemokines. Examples of chemokines are included in the specification. (See, e.g., page 1, lines 20-29; page 4, lines 18-32; page 12, lines 16-31; page 13, lines 1-2; and pages 44-45 (Table 1).) Many if not all of these active agents can be synthesized or purchased commercially from sources such as Pharmingen. The specification further teaches that full-length, truncated or elongated versions of chemokines may be used. (See page 14, lines 17-21.)

Second, the members of the genus commonly possess a biotin molecule conjugated to the pharmacologically active agent. The biotin molecule can be L biotin or the D isomeric form of biotin. The specification teaches that biotin suitable for use in the invention can be commercially obtained from Sigma Chemical Co. (See page 14, lines 8-10.)

Third, the members of the genus commonly possess a linker that conjugates the pharmacologically active agent to the biotin molecule. For active agents that are chemokines, the specification teaches that the linker is attached to the carboxyl terminus of the chemokine. (See page 14, lines 15-17.) Examples of these linker molecules are listed on pages 15, 16 and 46-49. Linkers can be synthesized or purchased from suppliers such as Pierce Chemical Co. Methods for linking the biotin

molecule to the active agent are also provided. (See page 16, lines 24-32, and page 17, lines 1-7, and Example 1.)

Fourth, the members of the genus commonly possess an anti-biotin antibody. (See page 17, lines 31-32, and page 18, lines 1-17, and Example 2.) These antibodies can be synthesized de novo or selected from anti-biotin antibodies commercially available from sources such as Vector Laboratories (Burlingame, CA) and Zymed Laboratories Inc. (South San Francisco, CA). Methods for screening antibodies in order to select those with a suitable association and dissociation rate are taught on page 18, lines 18-32, page19, lines 1-32, and page 20, lines 1-3, and Example 2. Anti-biotin antibodies can be conjugated to therapeutic or diagnostic agents such as those listed on pages 26 and 27.

Fifth, the complexes of the invention are able to bind to their cognate receptors in vivo or in vitro, and in doing so either stimulate, inhibit or simply label the receptor and preferably the cell on which it is expressed. Moreover, the complexes are able to dissociate in the presence of supra physiological levels of free biotin thereby allowing the active agent to interact with its receptor and potentially to be endocytosed, e.g., as part of a signaling pathway.

Accordingly, the specification provides to the ordinary artisan substantial guidance as to the common attributes or features of the genus of complexes of biotinylated active agents and anti-biotin antibodies. In view of the disclosureof the specification, the working examples including complexes of anti-biotin antibody with biotinylated ITAC or with biotinylated eotaxin (e.g., see Example 4b and 4d) combined with the knowledge and skill of one in the art are sufficient to satisfy the written description requirement for the genus claims 1 and 34.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1, 6, 7, 10, 11, 15, 20-24, 34, and 41-55, under 35 U.S.C. §112, first paragraph, for lack of written description.

## Rejection of Claims Under 35 U.S.C. §102

The Examiner has rejected claims 27 and 56-58 under 35 U.S.C. §102(e) as being anticipated by McCarty (USP 5,929,066).

Applicants have cancelled claim 27 and 56-58. Accordingly, Applicants respectfully request that the Examiner withdraw the rejection under 35 U.S.C. §102(e).

## Summary

Applicants believe that each of the pending claims now is in condition for allowance. Applicants respectfully request that the Examiner telephone the undersigned attorney in the event that the claims are not found to be in condition for allowance.

If the Examiner has any questions and believes that a telephone conference with Applicant's attorney would prove helpful in expediting the prosecution of this application, the Examiner is urged to call the undersigned at (617) 720-3500 (extension 343).

Respectfully submitted,

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DOCKET NO.: L0559/7001 ERP
~~DATE: August 10, 2001~~
**x08/10/01x**

**APPENDIX A**

**MARKED-UP CLAIMS**

1. A composition comprising:

(a) a biotin conjugate comprising:

(i) a biotin covalently coupled to

(ii) a pharmacologically active [agent]chemokine; and

(b) an anti-biotin antibody selectively bound to said biotin to form a complex.

6. (Cancelled)

7. (Cancelled)

10. The composition of claim 1, wherein the pharmacologically active [agent] chemokine has an agonist activity.

11. The composition of claim 1, wherein the pharmacologically active [agent] chemokine has an antagonist activity.

15. The composition of claim 1, wherein the complex has a half-life ranging from about 15 minutes to about 1 hour in the presence of supra physiological levels of biotin and an affinity constant ranging from about 1.0 to about 100.0 nanomolar.

20. The composition of claim 1, wherein the anti-biotin antibody comprises a therapeutic agent that is a cytotoxic agent.

21. The composition of claim 1, wherein the anti-biotin antibody comprises a diagnostic agent attached thereto.

22. The composition of claim 1, wherein the anti-biotin antibody has a dual specificity.

23. The composition of claim 22, wherein the anti-biotin antibody selectively binds to a tumor cell associated antigen.

24. The composition of claim 22, wherein the anti-biotin antibody selectively binds to a viral associated antigen.

27. (Cancelled)

34. A composition comprising:

(a) a biotin conjugate comprising

(i) a biotin covalently coupled to

(ii) [an agent] a chemokine having a pharmacological activity; and

(b) a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is suitable for parenteral administration.

41. The composition of claim 1, wherein the composition is lyophilized.

42. The composition of claim 1, further comprising a pharmaceutically acceptable carrier.

43. The composition of claim [3]42, wherein the pharmaceutically acceptable carrier is acceptable for a mode of delivery selected from the group consisting of: intradermal delivery, intramuscular delivery, intraperitoneal delivery, intravenous delivery, subcutaneous delivery, and controlled release delivery.

44. The composition of claim 1, wherein the biotin is selected from the group consisting of L-biotin, D-biotin and derivative thereof.

45. ~~The composition of claim [7]~~ 1, ~~wherein the chemokine is selected from the group~~ consisting of the chemokines of Table 1.

46. The composition of claim [7] 1, wherein the chemokine has a carboxyl terminus and the biotin is covalent attached to the carboxyl terminus of the chemokine.

47. The composition of claim 1, wherein the biotin is covalently coupled to the pharmacologically active [agent] chemokine via a linker molecule.

48. The composition of claim 1, wherein the complex has a half-life ranging from about 15 minutes to about 1 hour in the presence of supra physiological levels of biotin.

49. The composition of claim 1, wherein the anti-biotin antibody has an affinity constant ranging from about 1.0 to about 100.0 nanomolar.

50. The composition of claim 1, wherein the anti-biotin antibody is selected from the group consisting of an intact antibody, and an antibody fragment.

51. The composition of claim 1, wherein the anti-biotin antibody is a human antibody or fragment thereof.

52. The composition of claim 1, wherein the anti-biotin antibody has a subclass selected from the group consisting of a IgG1 subclass, and an IgG3 subclass.

53. The composition of claim 1, wherein the anti-biotin antibody comprises a therapeutic agent attached thereto.

54. The composition of claim 1, wherein the complex has a half-life of from one day to one month in vivo.

55. The composition of claim 1, wherein the complex has a half-life of from one week to two weeks in vivo.

56. (Cancelled)

57. (Cancelled)

58. (Cancelled)

# Appendix B

Appendix B

# G-protein coupled receptors and ligands as therapeutic agents

1: Drug Discov Today 1999 Feb;4(2):80-92

# Opportunities for novel therapeutic agents acting at chemokine receptors.

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Chemokines are proinflammatory mediators that primarily control leukocyte migration into selected tissues and upregulation of adhesion receptors. They also have a role in pathological conditions that require neovascularization and are implicated in the suppression of viral replication. By interaction with their respective G-protein-coupled receptor, chemokines have a profound influence over the selective recruitment of specific cell types in acute inflammatory disease and, hence, inhibition of their action should be of therapeutic benefit. Only now are small molecule inhibitors becoming available to validate this speculation. In this review, without seeking to be comprehensive, the authors provide an introduction to chemokines, their receptors and their role in certain disease processes. Also, recent disclosures claiming novel nonpeptide ligands for chemokine receptors are summarized.

PMID: 10234160 [PubMed - as supplied by publisher]

1: Blood Cells Mol Dis 1998 Mar;24(1):14-30

# Myeloid progenitor cell proliferation and mobilization effects of BB10010, a genetically engineered variant of human macrophage inflammatory protein-1alpha, in a phase I clinical trial in patients with relapsed/refractory breast cancer.

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ABSTRACT: Macrophage Inflammatory Protein (MIP)-1alpha is myelosuppressive in vitro and in vivo for hematopoietic stem and immature subsets of myeloid progenitor cells, demonstrates some myeloprotective effects in mice treated with Ara-C and hydroxyurea, and has stem/progenitor cell mobilizing activity in mice. Based on these observations, BB10010, a genetic variant of MIP-1alpha, was assessed for effects on marrow and blood myeloid progenitor cells in patients with relapsed/refractory breast cancer. MIP-1alpha readily polymerizes, whereas BB10010 has a reduced tendency to form large polymers at physiological pH and ionic strength and retains biological activity. Patients were injected with 5, 10, 30 or 100 &mgr;g/kg BB10010 s.c. daily for 3 days. BB10010 significantly reduced the cycling status of marrow myeloid progenitors from pretreatment levels of 39-58% to 0 - 11% one day after the third and last injection of BB10010. This was associated with significant decreases in frequency of marrow progenitors (number of colonies formed per number of cells plated) and percent biopsied marrow CD34+ cells. The suppressive effects were reversible in patients and the rapidity of this reversal demonstrated in mouse studies. BB10010 had no effect on nucleated cellularity or on the proliferation of nucleated cells as assessed in marrow biopsies from the patients. These latter effects may in part reflect the noted decreased apoptosis of nucleated cells by BB10010. BB10010 also demonstrated significant but modest myeloid progenitor cell mobilizing capacity. Blood progenitors were in a slow or non-cycling state prior to treatment and this did not change after administration of BB10010. The above effects of BB10010 were similar at the four different dosage levels assessed. These results demonstrate in humans the suppressive and mobilizing effects of MIP-1alpha and BB10010 previously noted in vivo in mice. 

Publication Types:
Clinical trial
Clinical trial, phase i

PMID: 9516378 [PubMed - indexed for MEDLINE]

1: Int Rev Immunol 1998;16(5-6):705-26

# Cytokines and chemokines in HIV infection: implications for therapy.

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HIV infection is associated with both a hyperactivity of the immune system and decreased immune responses against specific antigens. A similar pattern is observed when considering cytokine production in HIV-infected patients. Several cytokines are spontaneously produced at an increased level, whereas other cytokines playing an important role during cell-mediated immune responses are produced at a low level following stimulation. This deregulation of cytokine production may participate to the immune deficiency, both by impairing immune responses and by accelerating CD4+ T lymphocyte destruction. Chemokine receptors have recently been shown to function as coreceptors for the virus, and to govern its cellular tropism. Heterogeneous expression of chemokine receptor may contribute to differences in infectability as well as in rate of progression of the disease between individuals. Better understanding of the role of cytokines and chemokines in HIV infection suggests new therapeutic approaches where administration of cytokines or cytokine antagonists may allow the immune system to function in better conditions, to stimulate antiviral and antiinfectious immune defenses, and to limit viral spread.

Publication Types:
Review
Review, tutorial

PMID: 9646183 [PubMed - indexed for MEDLINE]

1: Dev Immunol 1998;6(1-2):3-11

Langerhans cell migration in murine cutaneous leishmaniasis: regulation by tumor necrosis factor alpha, interleukin-1 beta, and macrophage inflammatory protein-1 alpha.

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After intradermal infection of mice with the obligatory intracellular parasite Leishmania major, Langerhans cells (LC) are intimately involved in the induction of the primary T-cell immune response. LC can phagocytose Leishmania and transport ingested parasites from the infected skin to the regional lymph nodes. Since TNF alpha and IL-1 beta have been shown to induce LC migration after epicutaneous exposure to skin-sensitizing chemicals, we investigated the involvement of both cytokines in the migration of Leishmania-infected LC. In addition, the relevance of two chemokines of the beta subfamily, macrophage inflammatory protein 1 alpha (MIP-1 alpha) and macrophage chemoattractant protein 1 (MCP-1), was analyzed. In vivo depletion of TNF alpha significantly reduced the amount of infected LC and the parasite load in the draining lymph nodes. Administration of recombinant TNF alpha caused the reverse effect. In contrast, the depletion of IL-1 beta enhanced the parasite-induced LC migration, whereas treatment with recombinant IL-1 beta, as well as recombinant MIP-1 alpha, reduced the rate of infected LC in the lymph nodes. MCP-1 did not influence LC migration. Our data demonstrate that TNF alpha and IL-1 beta are regulating the LC-mediated transport of Leishmania and also provide evidence for the involvement of macrophage attractant chemokines in this process.

PMID: 9716900 [PubMed - indexed for MEDLINE]

1: Br J Pharmacol 1998 Aug;124(7):1369-74

# The intravenous administration of tumor necrosis factor alpha, interleukin 8 and macrophage-derived neutrophil chemotactic factor inhibits neutrophil migration by stimulating nitric oxide production.

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1. The i.v. administration of tumor necrosis factor-alpha (TNF-alpha), interleukin-8 (IL-8) and the recently described macrophage-derived neutrophil chemotactic factor (MNCF) inhibits the recruitment of neutrophils to the inflammatory site. 2. Pretreatment of mice with the NO synthase antagonist, NG-monomethyl-L-arginine (L-NMMA, 15-60 mg kg(-1)), but not the inactive enantiomer D-NMMA (30 mg kg(-1)), prevented in a dose-dependent manner the TNF-alpha, IL-8 and MNCF-mediated inhibition of neutrophil migration into thioglycollate-challenged peritoneal cavities. 3. Treatment of the neutrophils with TNFalpha (10(-7) M), IL-8 (10(-7) M) or MNCF blocked their migration towards FMLP in the chemotaxis assay. The pretreatment of the neutrophils with L-NMMA (50-200 microM) prevented in a dose-dependent manner the inhibition of FMLP-induced chemotaxis by IL-8, but did not alter the inhibition caused by TNF-alpha or MNCF. Different concentrations of the NO donors, S-nitroso-N-acetylpenicillamine (SNAP) or 3-morpholino-sydnonimine (SIN-1), did not alter this chemotaxis. 4. Preincubating the neutrophils with L-NMMA (200 microM) significantly increased the TNF-alpha (10(-7) M) and MNCF-mediated neutrophil adhesion to unstimulated endothelial cells, but had no effect on IL-8 (10(-7) M)-mediated adhesion. 5. Although NO donors did not directly affect the mechanisms of neutrophil motility, NO is involved in the in vitro inhibitory action of IL-8 on chemotaxis. The TNF-alpha and MNCF-mediated inhibition of neutrophil migration seems to be indirect, by affecting the mechanisms of adhesion. It was concluded that TNF-alpha-, IL-8- and MNCF-mediated inhibition of neutrophil migration is associated with the stimulation of NO production.

PMID: 9723947 [PubMed - indexed for MEDLINE]

1: Adv Exp Med Biol 1997;430:67-72

# Uncoupling of G-protein coupled receptors in vivo: insights from transgenic mice.

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Heart failure is a problem of increasing importance in medicine. An important characteristic of heart failure is reduced agonist-stimulated adenylyl cyclase activity (receptor desensitization) due to both diminished receptor number (receptor down regulation) and impaired receptor function (receptor uncoupling). These changes in the beta-adrenergic receptor (beta-AR) system, may in part account for some of the abnormalities of contractile function in this disease. Myocardial contraction is closely regulated by G-protein coupled beta-adrenergic receptors through the action of the second messenger cAMP. The beta-AR receptors themselves are regulated by a set of specific kinases, termed the G-protein-coupled receptor kinases (GRKs). The study of this complex system in vivo has recently been advanced by the development of transgenic and gene targeted ("knockout") mouse models. Combining transgenic technology with sophisticated physiological measurements of cardiac hemodynamics is an extremely powerful strategy to study the regulation of myocardial contractility in the normal and failing heart.

Publication Types:
Review
Review, tutorial

PMID: 9330719 [PubMed - indexed for MEDLINE]

1: Cell Signal 1998 May;10(5):313-20

# G protein-coupled receptor signalling in the kidney.

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The kidney is responsible for regulation of water and electrolyte balance, filtration and absorption of plasma proteins, and control of blood volume and pressure. Homeostasis achieved by the kidney is controlled in large part by the action of hormones or proteins on specific transmembrane receptors. Conversely, many renal diseases, including that resulting from atherosclerosis, are characterised by scarring and abnormal proliferation of cellular components of the kidney, and these processes are mediated in large part by these same receptors. The G protein-coupled receptors constitute a large and diverse class of proteins, characterised by the possession of seven transmembrane-spanning domains. These receptors bind polypeptide growth factors, which function to transmit a variety of signals from the extracellular to the intracellular milieu. The receptor-associated G proteins utilised by the kidney derive their specificity not only by activating or inhibiting various second-messenger molecules, but also by their location on particular cell types. In this review, several G protein-coupled receptors will be discussed from the perspective of their importance to kidney function and to the pathogenesis of renal disease, atherosclerosis, and hypertension.

Publication Types:
Review
Review, tutorial

PMID: 9692674 [PubMed - indexed for MEDLINE]

1: Eur J Neurosci 1999 Feb;11(2):427-32

# Intrathecal galanin alleviates allodynia-like behaviour in rats after partial peripheral nerve injury.

Hao JX, Shi TJ, Xu IS, Kaupilla T, Xu XJ, Hokfelt T, Bartfai T, Wiesenfeld-Hallin Z.

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We have previously suggested that the neuropeptides galanin and galanin message-associated peptide (GMAP) may have an inhibitory role in spinal nociception. The present study examined the effects of intrathecal (i.t.) administration of these two peptides on allodynia-like behaviours in response to mechanical and cold stimulation in rats after photochemically induced ischaemic peripheral nerve injury. I.t. galanin significantly alleviated the mechanical- and cold-allodynia-like behaviours in nerve injured rats, and was not associated with motor impairment or sedation. I.t. GMAP relieved mechanical allodynia much less than galanin. I.t. M-35, a high-affinity galanin receptor antagonist, did not significantly alter the response of the rats to mechanical or cold stimulation. At 1 or 2 weeks postinjury, around 15% of dorsal root ganglion (DRG) neuron profiles showed galanin-like immunoreactivity. These profiles were mostly small sized. Although the number of galanin positive cells was thus increased in the DRG in the present model, the increase was substantially less than after complete sciatic nerve section, as previously shown. The present results showed that spinal administration of galanin inhibited some abnormal pain-like behaviours in rats after partial peripheral nerve injury. These results further support an inhibitory function for galanin in nociception. However, endogenous galanin may not play a significant role in suppressing nociceptive input after partial ischaemic peripheral nerve injury, as the upregulation of galanin is moderate.

PMID: 10051743 [PubMed - indexed for MEDLINE]

1: Ann N Y Acad Sci 1998 Dec 21;863:143-55

# Role of galanin in the gastrointestinal sphincters.

Rattan S, Tamura W.

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Galanin was present and exerted potent effects in all the gastrointestinal sphincters examined. Galanin-immunoreactive nerve fibers and neurons are present in both the myenteric and submucosal plexuses of sphincters. The neuropeptide exerts diverse effects in different sphincteric smooth muscles that may be species specific. For example, in the lower esophageal sphincter, it may cause an increase in basal tone and suppression of nonadrenergic noncholinergic (NANC) nerve-mediated relaxation. On the contrary, in the internal anal sphincter (IAS), the predominant effect of galanin is to cause smooth muscle relaxation and augmentation of NANC nerve-mediated relaxation. In other sphincters, galanin may either have no effect or cause either an increase or a decrease in basal tone. Most of the actions of galanin on basal smooth muscle sphincteric tone are due to its actions directly on smooth muscle cells. However, some of the relaxant actions of the peptide may also be due to activation of NANC inhibitory neurons. The basic mechanism/s responsible for sphincteric smooth muscle contraction or relaxation in response to galanin have not been investigated. The suppressive as well as the augmentatory effects of galanin on NANC nerve-mediated sphincteric smooth muscle relaxation may be due to inhibition or facilitation, respectively, of the release of NANC inhibitory neurotransmitters such as nitric oxide and vasoactive intestinal polypeptide. Diverse effects in different gastrointestinal sphincters suggest a neuromodulatory rather than a neurotransmitter role of galanin and a significant role of the neuropeptide and putative antagonists in the pathophysiology and potential therapy of gastrointestinal motility disorders especially those affecting sphincteric function.

Publication Types:
Review
Review, tutorial

PMID: 9928167 [PubMed - indexed for MEDLINE]

1: Endocr Res 1998 Aug-Nov;24(3-4):835-43

# Abnormal expression and function of hormone receptors in adrenal Cushing's syndrome.

Lacroix A, N'Diaye N, Mircescu H, Hamet P, Tremblay J.

Department of Medicine, Research Center, Centre Hospitalier de l'Universite de Montreal, Canada.

The initial description of GIP-dependent Cushing's syndrome suggested that abnormal or ectopic expression of adrenal receptors for various ligands may underlie other cases of ACTH-independent hypercortisolism. GIP-dependent Cushing's syndrome has been described in patients with unilateral adenomas or bilateral ACTH-independent macronodular adrenal hyperplasia (AIMAH) and results from the adrenal overexpression of non-mutated GIP receptor. In AIMAH, other patients were identified in whom regulation of cortisol production resulted from an abnormal adrenocortical response either to vasopressin, beta-adrenergic receptor agonists, hCG/LH, or serotonin 5-HT-4 receptor agonists. The identification of the presence of an abnormal adrenal receptor offers the possibility of a new pharmacological approach to control hypercortisolism by suppressing the endogenous ligands or by using specific antagonists of the abnormal receptor.

Publication Types:
Review
Review, tutorial

PMID: 9888584 [PubMed - indexed for MEDLINE]

1: J Clin Endocrinol Metab 1998 Sep;83(9):3134-43

# Cushing's syndrome due to a gastric inhibitory polypeptide-dependent adrenal adenoma: insights into hormonal control of adrenocortical tumorigenesis.

Chabre O, Liakos P, Vivier J, Chaffanjon P, Labat-Moleur F, Martinie M, Bottari SP, Bachelot I, Chambaz EM, Defaye G, Feige JJ.

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We studied a patient with food-induced, ACTH-independent, Cushing's syndrome and a unilateral adrenocortical adenoma. In vivo cortisol secretion was stimulated by mixed, glucidic, lipidic, or proteic meals. Plasma ACTH levels were undetectable, but iv injection of ACTH stimulated cortisol secretion. Unilateral adrenalectomy was followed by hypocortisolism with loss of steroidogenic responses to both food and ACTH. In vitro, cortisol secretion by isolated tumor cells was stimulated by the gut hormone gastric inhibitory polypeptide (GIP) and ACTH, but not by another gut hormone, glucagon-like peptide-1 (GLP-1). Both peptides stimulated the production of cAMP but not of inositol 1,4,5-trisphosphate. In quiescent cells, GIP and ACTH stimulated [3H]thymidine incorporation and p42-p44 mitogen-activated protein kinase activity. GIP receptor messenger ribonucleic acid (RNA), assessed by RT-PCR, was highly expressed in the tumor, whereas it was undetectable in the adjacent hypotrophic adrenal tissue, in two adrenal tumors responsible for food-independent Cushing's syndrome, and in two hyperplastic adrenals associated with ACTH hypersecretion. In situ hybridization demonstrated that expression of GIP receptor RNA was confined to the adrenocortical tumor cells. Low levels of ACTH receptor messenger RNA were also detectable in the tumor. We conclude that abnormal expression of the GIP receptor allows adrenocortical cells to respond to food intake with an increase in cAMP that may participate in the stimulation of both cortisol secretion and proliferation of the tumor cells.

PMID: 9745416 [PubMed - indexed for MEDLINE]

1: Clin Exp Obstet Gynecol 1998;25(1-2):46-8

# A randomized trial of intracervical prostaglandin gel and intravenous oxytocin in prelabor rupture of membranes with unripe cervix at term.

Bilgin T, Kadioglu M, Yildirim V, Cengiz C.

Uludag University Faculty of Medicine, Department of Obstetrics and Gynecology, Bursa, Turkey.

In order to compare the efficacy of immediate intravenous oxytocin administration and intracervical prostaglandin E2 gel application in premature rupture of membranes with unfavorable cervices at term, 45 term pregnant patients with premature rupture of membranes were randomized into two groups. Twenty women received immediate intravenous oxytocin after cleansing enema while the rest were treated with intracervical prostaglandin E2 gel. Means of maternal age, gestational age, Bishop score at admission and the rates of nulliparity did not show any significant differences between the two groups ($p > 0.05$). The mean rupture to delivery time was 12.6 +/- 4.4 hours in the oxytocin group and 16.5 +/- 4.5 hours in the prostaglandin group ($p < 0.01$). Mean birth weights and Apgar scores were insignificant. Cesarean section rates were 24% in the oxytocin group and 5% in the other ($p < 0.05$). No infectious morbidity was seen in any case. In conclusion, although delivery is delayed with the intracervical prostaglandin approach, cesarean section rate is lowered without an increase in infectious morbidity.

Publication Types:
Clinical trial
Randomized controlled trial

PMID: 9743881 [PubMed - indexed for MEDLINE]

1: Biol Psychiatry 1999 Jan 15;45(2):145-57

# Oxytocin, vasopressin, and autism: is there a connection?

Insel TR, O'Brien DJ, Leckman JF.

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Autism is a poorly understood developmental disorder characterized by social impairment, communication deficits, and compulsive behavior. The authors review evidence from animal studies demonstrating that the nonapeptides, oxytocin and vasopressin, have unique effects on the normal expression of species-typical social behavior, communication, and rituals. Based on this evidence, they hypothesize that an abnormality in oxytocin or vasopressin neurotransmission may account for several features of autism. As autism appears to be a genetic disorder, mutations in the various peptide, peptide receptor, or lineage-specific developmental genes could lead to altered oxytocin or vasopressin neurotransmission. Many of these genes have been cloned and sequenced, and several polymorphisms have been identified. Recent gene targeting studies that alter expression of either the peptides or their receptors in the rodent brain partially support the autism hypothesis. While previous experience suggests caution in hypothesizing a cause or suggesting a treatment for autism, the available preclinical evidence with oxytocin and vasopressin recommends the need for clinical studies using gene scanning, pharmacological and neurobiological approaches.

Publication Types:
Review
Review, tutorial

PMID: 9951561 [PubMed - indexed for MEDLINE]

1: Clin Perinatol 1998 Dec;25(4):859-71, vi

# Oxytocin receptor antagonists. Update.

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For over three decades, scientists in a number of different laboratories have worked to design peptide analogues of oxytocin (OT) selective for the oxytocin receptor. Although there has been some interest in their use for treatment of dysmenorrhea, the principal clinical venue for such agents has been thought to lie in treatment of preterm labor. A major difficulty in identifying the clinical role for an OT antagonist had been our incomplete understanding of the role of OT in both term and preterm labor. This article begins with a review of the current understanding of the role of OT in the initiation and maintenance of labor in the human.

Publication Types:
Review
Review, tutorial

PMID: 9891619 [PubMed - indexed for MEDLINE]

Appendix B

# Labelled Antibodies

1: Br J Cancer 1997;75(6):822-8

# Immunoconjugates made of an anti-EGF receptor monoclonal antibody and type 1 ribosome-inactivating proteins from Saponaria ocymoides or Vaccaria pyramidata.

Di Massimo AM, Di Loreto M, Pacilli A, Rucci G, D'Alatri L, Mele A, Bolognesi A, Polito L, Stirpe F, De Santis R.

Menarini Ricerche SpA, Department of Biotechnology, Pomezia, Rome, Italy.

The present paper describes two immunoconjugates consisting of an anti-epidermal growth factor receptor (EGFR) monoclonal antibody (MAb), named Mint5, covalently linked to the type 1 ribosome-inactivating proteins (RIPs) ocymoidine (Ocy) and pyramidatine (Pyra) from Saponaria ocymoides and Vaccaria pyramidata respectively. Both antibody and toxins are shown to retain their respective biological properties upon chemical conjugation. The immunoconjugates exert specific inhibition of EGFR expressing target cell proliferation and protein synthesis in in vitro assays and also inhibit the growth of grafted human tumour cells in nude mice.

PMID: 9062402 [PubMed - indexed for MEDLINE]

1: Oncol Res 1992;4(11-12):447-53

# Enhanced antitumor activity of daunomycin conjugated with antigastric cancer monoclonal antibody MGb2.

Li S, Zhang XY, Qiao TD, Chen XT, Zhang SY, Chen LJ.

Laboratory of Gastroenterology, Xijing Hospital, Shaanxi, China.

In the present study, an antigastric cancer monoclonal antibody, MGb2, was chosen to prepare an antibody-daunomycin conjugate. Daunomycin was modified by cis-aconitic anhydride, and the derivative was linked to antibody, a carbodiimide reagent being used to produce peptide bonding. Four to five molecules of daunomycin were specifically bound per molecule of antibody, without severely impairing the pharmacological activity of daunomycin and with minimal loss of antibody activity. A tetrazolium dye colorimetric assay indicated that the MGb2-daunomycin conjugate exhibited selective cytotoxicity against human gastric cancer cells SGC-7901 in vitro. The tumor localization in BALB/c nude mice showed that the specific conjugate could recognize the tumor as efficiently as the unconjugated antibody. MGb2-daunomycin conjugate could significantly suppress the growth of human gastric carcinoma GAII inoculated under the renal capsules of BALB/c nude mice. Intraperitoneal injection of MGb2-daunomycin conjugate twice a week for 3 weeks at a dose of 1 mg/kg of drug gave a tumor inhibition rate of 91.58%, far more effective than free daunomycin or an irrelevant conjugate.

PMID: 1299375 [PubMed - indexed for MEDLINE]

1: Bioconjug Chem 1990 Jul-Aug;1(4):245-50

# Preparation of antigastric cancer monoclonal antibody MGb2-mitomycin C conjugate with improved antitumor activity.

Li S, Zhang XY, Zhang SY, Chen XT, Chen LJ, Shu YH, Zhang JL, Fan DM.

Laboratory of Gastroenterology, Xijing Hospital, Xi'an, Shaanxi Province, China.

In the present study, an antigastric cancer monoclonal antibody, MGb2, was chosen to prepare antibody-mitomycin C conjugate with dextran T-40 as intermediary. Up to 20 molecules of mitomycin C were specifically bound per molecule of antibody, without significantly impairing the antigen-binding capacity of the antibody and the pharmacological activity of mitomycin C. The conjugate showed selective cytotoxicity upon human gastric cancer cell line SGC-7901 in vitro. Radioimmunoimaging and biodistribution studies indicated that, after conjugation with mitomycin C via dextran T-40 as intermediary, the tumor localization capacity of the antibody was well-retained. When tested in nude mice inoculated with human gastric carcinoma GAII in bilateral subrenal capsules, intraperitoneal injection of the conjugate twice a week for 3 weeks at the dose of 1 mg/kg of drug gave a tumor inhibitory rate of 152.29%, the result being far better than that of free mitomycin C or an irrelevant conjugate. A similar result was found in another nude mouse model of human gastric carcinoma SGC-7901. Meanwhile, after conjugation with antibody, the toxicity of mitomycin C on tested animals was significantly reduced.

PMID: 2129014 [PubMed - indexed for MEDLINE]

1: Jpn J Cancer Res 1994 Feb;85(2):167-71

# In vivo efficacy of neocarzinostatin coupled with Fab human/mouse chimeric monoclonal antibody A7 against human colorectal cancer.

Yamaguchi T, Tsurumi H, Kotani T, Yamaoka N, Otsuji E, Kitamura K, Takahashi T.

First Department of Surgery, Kyoto Prefectural University of Medicine, Kyoto.

The anticancer polypeptide neocarzinostatin (NCS) was covalently coupled to a human/mouse chimeric Fab A7 monoclonal antibody (chFabA7) and the in vivo efficacy of this conjugate was examined. NCS concentration assay was carried out, and acute toxicity and tumoricidal effects were examined. The concentration assay, using anti-NCS monoclonal antibody, revealed that administration of the chA7Fab conjugate leads to a greater blood retention and a higher tumor accumulation of NCS, when compared to free NCS administration. The tumoricidal effect of chA7Fab-NCS was higher than that of either free NCS or the saline control, against antigen-positive tumors. In antigen-negative tumors there was no difference in toxic effect among the three preparations. Values of LD50, reflecting acute toxicity, were 5050 U/kg and 3600 U/kg for the chA7Fab-NCS and the free NCS, respectively. These results suggest that chFabA7-NCS may be a promising tool for targeting cancer chemotherapy.

PMID: 8144398 [PubMed - indexed for MEDLINE]

1: Semin Surg Oncol 1998 Dec;15(4):231-4

# Radioimmunoguided surgery with different iodine-125 radiolabeled monoclonal antibodies in recurrent colorectal cancer.

Percivale P, Bertoglio S, Meszaros P, Schenone F, Gipponi M, Moresco L, Cosso M, Badellino F.

Department of Clinical and Experimental Oncology, University of Genoa, Italy.

Sixty-four patients with recurrent or metastatic colorectal cancer underwent radioimmunoguided surgery (RIGS). Thirty patients (Group A) were preoperatively injected with radiolabeled monoclonal antibody (MAb) B72.3, a whole IgG1 that reacts with tumor-associated glycoprotein (TAG-72) antigen. Thirty-four patients (Group B) were given monoclonal antibody FO23C5, an F(ab')2 which reacts with the carcinoembryonic antigen (CEA). The use of F(ab')2 antibodies ensured a lower time interval from the preoperative injection of the radiolabeled MAb to surgery. This interval was 22.7 days for Group A patients and 10.9 days for Group B patients. The correct RIGS identification of tumor sites occurred in 80.4% of Group A patients and in 92.6% of Group B patients. Additional information capable of modifying surgical strategy was obtained in 23.3% of Group A patients and in 8.8% of Group B patients. This difference was due to the different patterns of biodistribution and pharmacokinetics of the two MAbs. Although FO23C5 yields an improved diagnostic resolution for macroscopic tumor sites, we believe that B72.3 or other whole IgG1 should be the first choice for RIGS in recurrent or metastatic colorectal cancer patients.

Publication Types:
Clinical trial
Controlled clinical trial

PMID: 9829378 [PubMed - indexed for MEDLINE]

1: J Nucl Med 1998 Nov;39(11):1937-43

# Delivery of therapeutic doses of radioiodine using bispecific antibody-targeted bivalent haptens.

Gautherot E, Le Doussal JM, Bouhou J, Manetti C, Martin M, Rouvier E, Barbet J.

Immunotech SA, Marseille, France.

Two-step pretargeting strategies have been designed to deliver radioisotopes to tumors more selectively than directly labeled antibodies or fragments. In this article, we compare quantitatively the potential of these strategies for the radioimmunotherapy of solid tumors. METHODS: Direct targeting was performed using iodine-labeled IgG and F(ab')2. As two-step strategies, we used the sequential injection of anti-CEA x anti-DTPA-In bispecific F(ab')2 (BsF(ab')2) and monovalent and bivalent DTPA derivatives labeled with iodine. The biodistribution of iodine in nude mice grafted with the LS174T human colorectal carcinoma was monitored in time and used for calculating radiation doses. RESULTS: In agreement with earlier studies, the IgG was more effective for delivering a radiation dose to the tumor than the F(ab')2 (7.8 versus 0.76 Gy/MBq, respectively) and both were moderately selective with respect to normal tissues (tumor:blood of 2.9 and 1.7, respectively). At their MTD, they should deliver 86 and 34 Gy, respectively, to the tumor. Using a nM-affinity DTPA-In bivalent hapten, the two-step protocol was optimized by varying the dosage of the BsF(ab')2, the stoichiometry of the reagents and the pretargeting time. The saturation of the tumor was obtained by injecting 5 nmol (500 microg) of BsF(ab')2. The pretargeted BsF(ab')2 was saturated by the injection of 0.5 mol of bivalent hapten per mole of antibody. With a 48-hr pretargeting time, the selectivity of the irradiation of the tumor was optimized (tumor:blood of 7.8) but only at the price of a lower efficiency (0.35 versus 0.86 Gy/MBq, 48-hr and 20-hr pretargeting time, respectively). Attempts to increase selectivity by using a microM-affinity DTPA-Y bivalent hapten or by chasing excess circulating radiolabeled hapten with an excess of unlabeled hapten also reduced tumor exposure. The use of a monovalent hapten resulted in both lower efficiency and selectivity. However, the two-step pretargeting of high-affinity bivalent hapten (Affinity Enhancement System, AES) should deliver 30-60 Gy to the tumor with less than 9 Gy to the blood in tumor-bearing mice. CONCLUSION: Radioimmunotherapy with AES is predicted to be as efficient and with lower hematological toxicity than direct targeting.

PMID: 9829586 [PubMed - indexed for MEDLINE]

1: Clin Cancer Res 1998 Nov;4(11):2729-39

# Phase I/II radioimmunotherapy trial with iodine-131-labeled monoclonal antibody G250 in metastatic renal cell carcinoma.

Divgi CR, Bander NH, Scott AM, O'Donoghue JA, Sgouros G, Welt S, Finn RD, Morrissey F, Capitelli P, Williams JM, Deland D, Nakhre A, Oosterwijk E, Gulec S, Graham MC, Larson SM, Old LJ.

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This Phase I/II radioimmunotherapy study was carried out to determine the maximum tolerated dose (MTD) and therapeutic potential of 131I-G250. Thirty-three patients with measurable metastatic renal cell carcinoma were treated. Groups of at least three patients received escalating amounts of 131I (30, 45, 60, 75, and 90 mCi/m2) labeled to 10 mg of mouse monoclonal antibody G250, administered as a single i.v. infusion. Fifteen patients were studied at the MTD of activity. No patient had received prior significant radiotherapy; one had received prior G250. Whole-body scintigrams and single-photon emission computed tomography images were obtained in all patients. There was targeting of radioactivity to all known tumor sites that were > or =2 cm. Reversible liver function test abnormalities were observed in the majority of patients (27 of 33 patients). There was no correlation between the amount of 131I administered or hepatic absorbed radiation dose (median, 0.073 Gy/mCi) and the extent or nature of hepatic toxicity. Two of the first six patients at 90 mCi/m2 had grade > or =3 thrombocytopenia; the MTD was determined to be 90 mCi/m2 131I. Hematological toxicity was correlated with whole-body absorbed radiation dose. All patients developed human antimouse antibodies within 4 weeks posttherapy; retreatment was, therefore, not possible. Seventeen of 33 evaluable patients had stable disease. There were no major responses. On the basis of external imaging, 131I-labeled mouse monoclonal antibody G250 showed excellent localization to all tumors that were > or =2 cm. Seventeen of 33 patients had stable disease, with tumor shrinkage observed in two patients. Antibody immunogenicity restricted therapy to a single infusion. Studies with a nonimmunogenic G250 antibody are warranted.

Publication Types:
Clinical trial
Clinical trial, phase i
Clinical trial, phase ii

PMID: 9829736 [PubMed - indexed for MEDLINE]

1: Zhonghua Fu Chan Ke Za Zhi 1994 May;29(5):296-8, 319

# [Target therapy by monoclonal antibody against ovarian carcinoma conjugated with liposome and adriamycin].

[Article in Chinese]

Li WJ, Qian HN, Lu WY.

Beijing Medical University People's Hospital Gynecology Oncology Research Laboratory.

Conjugates of monoclonal antibody (MAb) and adriamycin entrapped in liposome (MLA) were prepared by COC166-9. The MAb against ovarian serous adenocarcinoma was generated in our laboratory. Target therapies were done in nude mice model with subcutaneous tumor xenografts in 24 and ascitic carcinoma in 16 by MLA. The results demonstrated that MLA group presented the best therapeutic effect than all the other groups, which gave a very helpful clue to clinical target therapy of ovarian carcinoma in the future.

PMID: 7956556 [PubMed - indexed for MEDLINE]

1: Clin Cancer Res 1998 Nov;4(11):2691-700

# Phase I study of the pharmacokinetics of a radioimmunoconjugate, 90Y-T101, in patients with CD5-expressing leukemia and lymphoma.

Foss FM, Raubitscheck A, Mulshine JL, Fleisher TA, Reynolds JC, Paik CH, Neumann RD, Boland C, Perentesis P, Brown MR, Frincke JM, Lello CP, Larson SM, Carrasquillo JA.

National Cancer Institute Navy Medical Oncology Branch, National Cancer Institute, Bethesda, Maryland 20889, USA.

Ten patients with advanced or refractory CD5-expressing hematologic neoplasms [two with chronic lymphocytic leukemia and eight with cutaneous T-cell lymphoma (CTCL)] were treated in a Phase I study with the radioimmunoconjugate 90Y-T101, which targets CD5+ lymphocytes. Prior imaging studies using 111In-T101 demonstrated uptake in involved lymph nodes and skin in patients with CTCL, and Phase I studies with unmodified T101 demonstrated transient responses. In this study, patients were treated with 5 or 10 mCi of 90Y chelated to T101 via isothiocyanatobenzyl diethylenetriamine pentaacetic acid, along with tracer doses of 111In-T101 for imaging. The biodistribution of the radioimmunoconjugate was determined by measuring 90Y and 111In blood clearance, urine excretion, and accumulation in bone marrow and in involved skin lesions. The intravascular pharmacokinetics of 90Y were predicted by 111In-labeled T101. The greatest differences in biodistribution between 111In and 90Y were in the higher bone accumulation of 90Y and its lower urinary excretion. Imaging studies demonstrated targeting of skin lesions and involved lymph nodes in CTCL patients. The predominant toxicity was bone marrow suppression. Rapid antigenic modulation of CD5 on circulating T and B cells was observed. Recovery of T-cell populations occurred within 2-3 weeks; however, suppression of B-cell populations persisted after 5+ weeks. All CTCL patients developed human antimouse antibody after one cycle and thus were not retreated; one patient with chronic lymphocytic leukemia received a second cycle of therapy. Partial responses occurred in five patients, two with chronic lymphocytic leukemia and three with CTCL. The median response duration was 23 weeks. One CTCL patient who subsequently received electron beam irradiation to a residual lesion is disease-free after 6 years.

Publication Types:
Clinical trial
Clinical trial, phase i

PMID: 9829731 [PubMed - indexed for MEDLINE]

1: Anat Rec 1996 Aug;245(4,:652-61

# Antibody immunoglobulin G (IgG) against human prostatic specific antigen (PSA) as a carrier protein for chemotherapeutic drugs to human prostate tumors: Part 1. A double immunofluorescence analysis.

Sinha AA, Sackrison JL, DeLeon OF, Wilson MJ, Gleason DF.

Research Service, Veterans Affairs Medical Center, Minneapolis, MN 55417, USA.

BACKGROUND: Adenocarcinoma of the prostate (CaP) is the the second highest cause of cancer deaths in U.S. males. Current chemotherapeutic and/or endocrine treatments do not specifically and selectively target tumor cells of prostate cancer and benign prostatic hyperplasia (BPH). We hypothesized that because of the specific binding characteristics of antibody immunoglobulin G (IgG) to human prostatic-specific antigen (PSA), PSA-IgG could function as a carrier protein for conjugated chemotherapeutic drugs and that the immunoconjugate would selectively bind to prostatic epithelial cells and their tumors, but not to epithelial cells of unrelated organs. Our objective was to test the hypothesis using human prostatectomy specimens. METHODS: WE used several derivatives of 5'-fluorouracil, namely, 5'-fluoro- 2'-deoxyuridine (5'-Fu-2'-d), 5'-fluoro-2'-deoxyuridine-5' monophosphate (5'-Fu-2'-d-5'-mp), 5'-fluoro-2'-deoxyuridine-5'-(p-aminophenyl) monophosphate (5'-Fu-2'-d'-5'-amp), to conjugate with rabbit anti-PSA-IgG together with fluorescent markers (such as rhodamine and fluorescein or fluorescein isothiocyanate: FITC). Prostate specimens were obtained from prostatectomy patients who had not been treated with cytotoxic drugs before surgery. We evaluated formalin-fixed and paraffin-embedded sections as well as cryostat sections of frozen specimens for localization of PSA-IgG alone and PSA-IgG-drug immunoconjugate using immunoperoxidase (IP) and single and/or double immunofluorescence (IF) localization techniques. RESULTS: Our study showed that the immunoconjugate (PSA-IgG-5'-Fu-2'-d) bound to PSA (molecular size of approximately 34 KDa) on nitrocellulose sheets in Western immunoblots of extracts of BPH and CaP tissues. This binding of immunoconjugate to PSA on immunoblots was similar to that of the unconjugated PSA-IgG. Immunostaining patterns for rabbit anti-PSA-IgG and PSA-IgG-5'-Fu-2'-d immunoconjugate were similar and specific for prostate epithelial cells and their tumors, as revealed by IP techniques. To demonstrate that both the antibody and drug localized in the same group of prostatic epithelial cells, we used an immunoconjugate in which the PSA-IgG was labeled with rhodamine and 5'-Fu-2'-d-5'-amp with FITC. Our study showed that fluorescence for rhodamine and FITC was present in the same group of prostatic epithelial cells. Phase contrast microscopy demonstrated details of prostatic glandular epithelium and connective tissues. Our study showed that fluorescence for rhodamine and FITC and immunostaining by IP techniques were not observed in prostate sections incubated with normal rabbit serum. CONCLUSIONS: We have shown that conjugation of 5'-Fu derivatives to PSA-IgG did not affect either the selectivity or specificity of the antibody for prostatic epithelial cells. Differential immunofluorescence study has shown that PSA-IgG may function as a carrier protein for chemotherapeutic drugs to prostate epithelial cells and their tumors. Furthermore, FITC-labeled 5'-Fu-2'-d did not specifically localize in prostatic glands, kidney, lungs, bladder, or colon. Because of the specificity and selectivity of the immunoconjugate for prostatic epithelial cells and their tumors, the immunoconjugate could be used in small dosages to treat prostatic tumors and such treatment would greatly reduce many unpleasant side effects in patients. This is the first report to show that PSA-IgG can function as an organ specific carrier protein for chemotherapeutic drugs to human prostate epithelium and its tumors.

PMID: 8837723 [PubMed - indexed for MEDLINE]

1: Chin Med Sci J 1994 Jun;9(2):75-80

# Antitumor activity of immunoconjugates composed of boanmycin and monoclonal antibody.

Zhen Y, Peng Z, Deng Y, Xu H, Chen Y, Tian P, Li D, Jiang M.

Institute of Medicinal Biotechnology, CAMS & PUMC, Beijing.

Boanmycin (bleomycin A6, BM), an antitumor antibiotic, was conjugated to monoclonal antibodies including R19, H111 and CCT2. The immunoconjugates exhibited selective cytotoxicity to related target cells including cecum cancer Hce-8693 cells, liver cancer BEL-7402 cells and leukemia CEM cells. They were highly effective against related human tumor xenografts in nude mice, and the inhibition rates by the conjugates were much higher than those by free BM. The inhibition rate by R19-BM conjugate against human cecum cancer xenografts reached 90%. BY immunoelectron microscopy, CCT2-BM conjugate showed specific binding and internalization in leukemia CEM cells. The results indicate that boanmycin-monoclonal antibody immunoconjugates are highly active both in vitro and in vivo.

PMID: 7528068 [PubMed - indexed for MEDLINE]

Appendix B

# Bispecific Antibodies

1: Farmaco 1997 Feb;52(2):71-81

# In vitro and in vivo antitumor activity of immunoconjugates prepared by linking 5-fluorouridine to antiadenocarcinoma monoclonal antibody.

Brusa P, Dosio F, Coppo S, Pacchioni D, Arpicco S, Crosasso P, Cattel L.

Dipartimento di Scienza e Tecnologia del Farmaco, Torino, Italy.

5-Fluorouridine (5-FUr), a cytotoxic antitumoral agent not in clinical use because of its systemic toxicity, and AR-3, a monoclonal antibody specific to a human colorectal adenocarcinoma, were covalently linked via two different strategies. 5-FUr was 5' succinilated after protection of the secondary hydroxyl groups and the carboxylate derivative was then activated as N-hydroxysuccinimidyl ester in order to react with the amino groups present in the monoclonal antibody, giving an amide linkage. Alternatively, a 5-FUr immunoconjugate containing an acid-cleavable hydrazone bond was formed from the reaction between an acyl hydrazide derivative of 5-FUr and a periodate oxydized antibody with approximately 12 aldehyde groups in its carbohydrate region. An average of 9 to 12 drug molecules were attached to the antibody. In a cytotoxic assay on the human colorectal carcinoma cell line HT-29, the hydrazone containing drug conjugate was equally active as the succinylamido conjugate and the free drug. However, ELISA showed that while in the case of the succinylamido conjugate the Mab immunoreactivity was not affected after conjugation, there was a significant loss of reactivity in the acid cleavable conjugate. In a model of a disseminated intraabdominal carcinomatosis by HT-29 intraperitoneal graft in nude mice, the 5-FUr immunoconjugate selected was more effective than the unconjugated drug in medium-term therapy (21 days after the graft and 16 days after drug treatment), albeit in the longer period the efficacy of the two formulations was similar. The toxic effect of the drug-conjugate in vivo was much weaker, demonstrating its clear advantage over the drug, in terms of pharmacological efficacy.

PMID: 9181686 [PubMed - indexed for MEDLINE]

1: Cancer Res 1997 Sep 15;57(18):4008-14

# Bispecific antibody-dependent cellular cytotoxicity of HER2/neu-overexpressing tumor cells by Fc gamma receptor type I-expressing effector cells.

Keler T, Graziano RF, Mandal A, Wallace PK, Fisher J, Guyre PM, Fanger MW, Deo YM.

Medarex, Inc., Annandale, New Jersey 08801, USA.

A bispecific antibody, MDX-H210, was developed to target cytotoxic effector cells expressing Fc gamma receptor type I (Fc gammaRI, CD64) to HER2/neu-overexpressing tumor cells. HER2/neu is an appropriate target for immunotherapy due to the high level of expression of this proto-oncogene in a variety of malignancies. The expression of Fc gammaRI is limited primarily to cytotoxic immune cells, including monocytes, macrophages, and cytokine-activated polymorphonuclear (PMN) cells. Therefore, tumor cells bound with MDX-H210 can be selectively recognized by effector cells with cytotoxic potential. MDX-H210 was prepared by chemical conjugation of Fab' fragments derived from the HER2/neu-specific monoclonal antibody, 520C9, and the Fc gammaRI-specific monoclonal antibody, H22. This bispecific molecule demonstrated specific, dose-dependent, and saturable binding to both HER2/neu- and Fc gammaRI-expressing cells. A solid-phase immunoassay that demonstrated simultaneous and specific binding to both antigens was used to confirm the bispecific nature of MDX-H210. Monocytes and PMN cells mediated MDX-H210-dependent lysis of HER2/neu-overexpressing cell lines derived from breast, ovarian, and lung carcinomas. IFN-gamma treatment of monocytes enhanced antibody-dependent cellular cytotoxicity, whereas IFN-gamma and granulocyte colony-stimulating factor were required for PMN cell-mediated tumor cell lysis. In addition, MDX-H210 elicited tumor necrosis factor-alpha secretion from monocytes when cultured in the presence of HER2/neu-positive target cells. These in vitro data suggest that targeting tumor cells to Fc gammaRI with MDX-H210 may be an effective treatment for malignancies that overexpress HER2/neu. The in vivo cytotoxic potential of MDX-H210 may be enhanced by combination therapy with the cytokines granulocyte colony-stimulating factor and IFN-gamma, which up-regulate Fc gammaRI expression on cytotoxic effector cells.

PMID: 9307286 [PubMed - indexed for MEDLINE]

1: Cancer Immunol Immunother 1997 Nov-Dec;45(3-4):162-5

# Bispecific antibody treatment of murine B cell lymphoma.

De Jonge J, Heirman C, De Veerman M, Van Meirvenne S, Demanet C, Brissinck J, Thielemens K.

Laboratory of Physiology, Medical School, Vrije Universiteit Brussel, (VUB), Belgium.

This report summarizes our experimental data concerning the use of bispecific antibodies (bsAb) for the treatment of the murine BCL1 B cell lymphoma model. Initially we used a hybrid-hybridoma-derived bsAb with specificity for the TcR/CD3 complex on T cells and the idiotype of the membrane-bound IgM on the tumor cells. The bsAb used as a single agent could cure animals with a low tumor load (resembling minimal residual disease). However, in experiments aimed at increasing the therapeutic effect in animals with a higher tumor burden, we could demonstrate the importance of additional T-cell-costimulatory signals and the careful timing of the bsAb administration. Recently we have generated a bispecific single-chain Fv (bsscFv) fusion protein with the same dual specificity as the hybrid-hybridoma-derived bsAb. Immunotherapy with this smaller molecule also resulted in tumor elimination in BCL1-bearing mice. A second bsscFv (alpha-CD19 x alpha-CD3) with a broader applicability is now being characterized and tested in vivo.

PMID: 9435864 [PubMed - indexed for MEDLINE]

1: J Immunol 1997 Aug 1;159(3):1543-56

# Bispecific humanized anti-IL-2 receptor alpha beta antibodies inhibitory for both IL-2- and IL-15-mediated proliferation.

Pilson RS, Levin W, Desai B, Reik LM, Lin P, Korkmaz-Duffy E, Campbell E, Tso JY, Kerwin JA, Hakimi J.

Roche Research Center, Inflammation and Autoimmune Diseases, Hoffmann-La Roche, Inc., Nutley, NJ 07110, USA.

Humanized anti-Tac (HAT) and Mik beta1 (HuMik beta 1) Abs directed at IL-2R alpha and IL-2R beta, respectively, inhibit IL-2 binding and biological activity and together act synergistically in vitro. The Abs have been used successfully in primate models of allograft rejection, graft-vs-host disease, and autoimmunity. We produced bifunctional humanized anti-IL-2R alpha beta Abs (BF-IgG) to combine the specificity of the two Abs into one entity by fusing HAT-producing NSO cells and HuMik beta 1-producing Sp2/0 cells. BF-IgG was purified using protein G-Sepharose affinity chromatography, followed by IL-2R alpha and IL-2R beta affinity chromatography and hydrophobic interaction chromatography. BF-IgG exhibited both anti-IL-2R alpha and anti-IL-2R beta specificities in binding assays. While the Ab binds the IL-2R with intermediate affinity (Kd = 2.82 nM), it does not inhibit IL-15 binding to its high affinity IL-15R. In Kit225/K6 (IL-2R alpha beta gamma+) cells, BF-IgG was 10-fold more potent than a HAT/HuMik beta 1 equimolar mixture in blocking IL-2-induced proliferation and, unexpectedly, was at least 65-fold more active than the mixture in blocking IL-15-induced proliferation. This dual inhibitory activity may be due to cross-linking of the IL-2R alpha and IL-2R beta, thus blocking IL-2 binding and possibly impeding the association of IL-2R beta with IL-15R. BF-IgG has potent immunosuppressant activities against both IL-2- and IL-15-mediated responses, and this antagonist could be more efficacious than HAT and/or HuMik beta 1 for the treatment of autoimmunity and the prevention of allograft rejection.

PMID: 9233654 [PubMed - indexed for MEDLINE]

1: Br J Cancer 1996 Mar;73(6):721-7

# The role of apoptosis in bispecific antibody-mediated T-cell cytotoxicity.

Kroesen BJ, Wellenberg GJ, Bakker A, Helfrich W, The TH, de Leij L.

Department of Clinical Immunology, University Hospital Groningen, The Netherlands.

In this report we describe the role of apoptosis in the process of tumour cell killing by bispecific monoclonal antibody (BsMAb)-redirected cytolytic T cells. The BsMAb used, BIS-1, has dual specificity for the CD3 complex on T cells and the pancarcinoma-associated 38 kDa transmembrane antigen EGP-2. BIS-1 allows activated T cells to specifically recognise and kill EGP-2-positive but not EGP-2-negative target cells. An assay was developed to quantify apoptosis in cells by separation of 3H-thymidine-labelled low-molecular, i.e. fragmented, from high-molecular, i.e. non-fragmented DNA. The presence of low molecular weight DNA was measured both within the target cells and in the cell-free supernatant. After exposure to BIS-1-redirected, -activated T cells, apoptosis was observed in EGP-2-positive target cells but not in EGP-2-negative target cells. Also no DNA fragmentation proved to be induced in the activated effector cells during assay. The degree of EGP-2-positive target DNA fragmentation depended on the concentration of BsMAb, the E/T ratio and the incubation time. Using a low E/T ratio (1/1), DNA fragmentation in and 51Cr release from target cells showed similar characteristics and kinetics. At higher E/T ratio (20/1), the 51Cr release from the target cells increased to a greater extent than the percentage fragmented target cell DNA. Inhibitors of DNA fragmentation added to the cytotoxicity assay inhibited not only DNA fragmentation, but also the release of chromium-51 from the target cells, suggesting that apoptosis and cell lysis are closely related in BsMAb-mediated cell killing.

PMID: 8611371 [PubMed - indexed for MEDLINE]

1: Bioconjug Chem 1996 Sep-Oct;7(5):532-5

# Bispecific antibody mediated targeting of nido-carboranes to human colon carcinoma cells.

Primus FJ, Pak RH, Richard-Dickson KJ, Szalai G, Bolen JL Jr, Kane RR, Hawthorne MF.

Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, California 91010, USA.

Boron neutron capture therapy, a binary form of cancer treatment, has the potential to deliver potent cytotoxic radiation to tumor cells with minimal collateral damage to normal tissues if methods for the selective accretion of elevated concentrations of boron-10 in tumor can be developed. In this regard, a monoclonal antibody with dual specificity, for both anionic boron cluster compounds (nido-carboranes) and a tumor-associated antigen (carcinoembryonic antigen, CEA), was produced. The specific binding of a nido-carborane to CEA-expressing tumor cells was achieved using this bispecific antibody. The ability of this bispecific antibody to concentrate selectively at tumor sites in vivo has also been demonstrated, thus suggesting its potential for sequestering boron-rich compounds in tumors.

PMID: 8889012 [PubMed - indexed for MEDLINE]

## Treatment of Hodgkin's disease with bispecific antibodies.

**Hartmann F, Renner C, Jung W, Sahin U, Pfreundschuh M.**

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Bispecific monoclonal antibodies (Bi-MAbs) with dual specificity for tumor-associated antigens (TAA) and a triggering molecule of an immunologic effector cell, respectively, open the possibility to specifically target to and activate cytotoxic effector cells (macrophages, T-cells, NK cells) at the tumor site. Using appropriately designed Bi-MAbs and unstimulated human NK cells and T-cells, respectively, we were able to cure SCID mice xenografted with human Hodgkin's tumors. This approach was also effective in disseminated tumors and when treatment was delayed until three weeks after the inoculation of the tumor, thus establishing this approach as the most effective model of an immunomodulating therapy of human neoplasms. Early observations with an ongoing phase I/II study with CD16/CD30 Bi-MAb in patients with refractory Hodgkin's disease confirm the expected low toxicity. If these observations can be confirmed in larger clinical studies, effector cell activating Bi-MAbs could become an important weapon in the remaining fight for the conquest of Hodgkin's disease.

Publication Types:
- Review
- Review, tutorial

PMID: 8836426 [PubMed - indexed for MEDLINE]

1: Mol Immunol 1995 Dec;32(17-18):1405-12

# Production and characterization of bispecific single-chain antibody fragments.

De Jonge J, Brissinck J, Heirman C, Demanet C, Leo O, Moser M, Thielemans K.

Laboratory of Physiology, Medical School, Vrije Universiteit, Brussels, Belgium.

We report the construction, expression and purification of a bispecific single-chain Fv antibody fragment produced in Escherichia coli. The protein possesses a dual specificity: the single-chain FvB1 portion is directed to the Idiotype of BCL1 lymphoma cells, the single-chain Fv2C11 moiety binds to the CD3 marker on T cells. The two domains are joined by a flexible peptide linker. Using Immobilized Metal Affinity Chromatography, the recombinant protein was purified from bacterial insoluble membrane fractions. After refolding of the bispecific protein, it was affinity-purified. As demonstrated by flow cytometry, both binding sites are retained in the refolded protein. Retargeted cytotoxicity and T cell proliferation assays further prove the biological activity and specificity of the bispecific single-chain Fv. Thus, these bispecific molecules show a potential anti-tumor activity.

PMID: 8643110 [PubMed - indexed for MEDLINE]

# Appendix B

## Agonists and Antagonists for G-protein coupled receptors and Ligands (See also receptors and Ligands as therapeutic agents)

1: J Immunol 1991 Aug 1;147(3):883-92

# Intravascular IL-8. Inhibitor of polymorphonuclear leukocyte accumulation at sites of acute inflammation.

Hechtman DH, Cybulsky MI, Fuchs HJ, Baker JB, Gimbrone MA Jr.

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IL-8 has been characterized primarily as a polymorphonuclear leukocyte (PMN) chemoattractant and proinflammatory mediator. Recently, we have reported that [Ala-IL-8]77 is secreted by activated cultured human endothelial cells and can function as a potent inhibitor of PMN adhesion to these monolayers. The pathophysiologic relevance of this in vitro observation was examined by determining the effects of intravascular or extravascular administration of IL-8 on PMN emigration at sites of acute inflammation in the skin of NZW rabbits. An i.v. bolus of [Ala-IL-8]77 (12 micrograms/kg) produced a marked and selective reduction of circulating PMN within 3 min, which returned toward preinjection levels within 30 min, and subsequently exceeded this level. A similar response was observed for circulating radiolabeled PMN, and gamma-scintigraphy determined that the lungs were the primary site of leukosequestration. During the 30- to 150-min interval after i.v. infusion of [Ala-IL-8]77, PMN emigration into acute inflammatory sites, elicited by various chemoattractants or cytokines, was significantly reduced, as judged histologically and quantitated with 51Cr-labeled PMN and myeloperoxidase measurements. Intravenous administration of [Ser-IL-8]72 yielded similar results. This inhibitory effect of i.v. IL-8 was transient and reinducible and did not reflect a suppression of the responsiveness of circulating PMN to chemoattractants. Intradermal injections of [Ala-IL-8]77 or [Ser-IL-8]72 induced dose-dependent PMN accumulation, which also was significantly reduced by i.v. administration of either form of IL-8. These results indicate that i.v. IL-8 can function as a PMN-directed leukocyte adhesion inhibitor and suggest that local secretion of IL-8 by activated endothelium may differentially modulate leukocyte-endothelial interactions at sites of acute inflammation.

PMID: 1650387 [PubMed - indexed for MEDLINE]

1: Int J Cancer 1993 Dec 2;55(6):963-7

# Effect of somatostatin analog RC-160 and bombesin/gastrin releasing peptide antagonist RC-3095 on growth of PC-3 human prostate-cancer xenografts in nude mice.

Pinski J, Schally AV, Halmos G, Szepeshazi K.

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Nude mice bearing xenografts of the androgen-independent human prostate-cancer cell line PC-3 were treated for 4 weeks with somatostatin analog RC-160, bombesin/gastrin-releasing peptide (GRP) antagonist (RC-3095), or the combination of both peptides. In the first experiment, treatment was started when the tumors measured approximately 10 mm3. Tumor volumes and weights were reduced by about 40% by RC-160 or RC-3095 administered by s.c. injections at doses of 100 micrograms/day/animal and 20 micrograms/day/animal respectively. The combination of RC-3095 with RC-160 did not further potentiate suppression of tumor growth, but histologically the ratio of apoptotic and mitotic indices was significantly higher in the groups treated with the combination than in the other groups. Serum gastrin levels were significantly reduced in all treated groups. Therapy with RC-160 or the combination also significantly decreased serum growth-hormone levels. Specific high-affinity binding sites for bombesin, somatostatin and epidermal growth factor (EGF) were found on the tumor membranes. Receptors for EGF were significantly down-regulated by treatment with RC-3095, RC-160 and a combination of both analogs. Tumors from mice treated with RC-160 showed a significant increase in maximal binding capacity for somatostatin as compared with control tumors, demonstrating the absence of down-regulation. In the second experiment, treatment was started when the tumors were well developed and measured approximately 90 mm3. No significant reduction in volume, weight and growth rate of tumors was found in the groups treated with RC-160 or RC-3095. Our results suggest that somatostatin analog RC-160 and bombesin/GRP antagonist RC-3095 can inhibit the growth of androgen-independent prostate cancer when the therapy is started at an early stage of tumor development.

PMID: 7902829 [PubMed - indexed for MEDLINE]

1: Cancer 1993 Dec 1;72(11):3263-70

# Antagonists of bombesin/gastrin-releasing peptides as adjuncts to agonists of luteinizing hormone-releasing hormone in the treatment of experimental prostate cancer.

Pinski J, Halmos G, Szepeshazi K, Schally AV.

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BACKGROUND. Palliative methods for treatment of advanced prostatic carcinoma, including those based on luteinizing hormone-releasing hormone (LH-RH) agonists, cannot prevent the ultimate growth of hormone-independent cells, and the duration of disease remission in patients with prostate cancer is limited. New therapeutic approaches combining androgen ablation therapy with other compounds must be explored. Various studies suggest that bombesin or gastrin-releasing peptide (GRP) act as autocrine growth factors and may play a role in the initiation and progression of some cancers, including those of the prostate. METHODS. The effects of treatment with bombesin/gastrin-releasing peptide (GRP) receptor antagonist [D-Tpi6, Leu13 psi(CH2NH)Leu14]BN(6-14)(RC-3095), an agonist of LH-RH [D-Lys6]-LH-RH and their combination were investigated in the androgen-dependent Dunning R-3327H rat prostate cancer model. Both analogs were administered by continuous subcutaneous infusion from osmotic minipumps for 7 weeks. RESULTS. Tumor volumes and weights were significantly reduced by treatment with RC-3095, compared with those of controls. In rats that received [D-Lys6]-LH-RH, there was a greater decrease in tumor weight and volume than that produced by RC-3095, and the weights of testes, ventral prostate, and seminal vesicles also were reduced. The combination of RC-3095 and [D-Lys6]-LH-RH had the greatest inhibitory effect on tumor growth. Histologic parameters demonstrated a significant increase of the ratio of apoptotic to mitotic indices in the groups treated with [D-Lys6]-LH-RH or the combination. Serum LH and testosterone levels were greatly depressed by [D-Lys6]-LH-RH or the combination. Specific high-affinity binding sites for bombesin/GRP, epidermal growth factor (EGF), and insulin-like growth Factor I (IGF-I) were found on the tumor membranes. The concentration of receptors for EGF was significantly reduced by treatment with the bombesin/GRP antagonist RC-3095. CONCLUSIONS. Combination therapy of LH-RH analogs with bombesin antagonists such as RC-3095 might be considered for improvement of hormonal therapy of prostate cancer.

PMID: 8242552 [PubMed - indexed for MEDLINE]

1: Brain Res 1993 Jun 18;614(1-2):125-30

# Stimulatory effects of bombesin-like peptides on suprachiasmatic neurons in brain slices.

Tang KC, Pan JT.

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The effects of bombesin on hypothalamic suprachiasmatic (SCN) neurons were tested in this study using extracellular single-unit recording in brain tissue slices. Fresh slices containing the SCN were obtained from adult ovariectomized Sprague-Dawley rats. Bombesin in pmol ranges stimulated 75% of irregular firing SCN neurons (n = 113), while it stimulated and inhibited 17% and 34% of regular firing units, respectively. Half of the regular firing SCN units, however, were not responsive to bombesin (49% of 61 units). A dose-dependent (from 5 to 500 pmol) excitatory effect of bombesin on SCN neurons was also observed. Pretreatment with [Leu13-psi(CH2NH)-Leu14]-bombesin, a bombesin receptor antagonist, blocked the action of bombesin in 67% of 18 units responded to bombesin, indicating a specific receptor is involved in the action. Gastrin-releasing peptide, a well-recognized bombesin-like peptide in mammals, behaved almost the same as bombesin did in most SCN neurons tested (same responses in 24 of 25 units). The present finding indicates that bomesin-like peptides may play a significant role in the SCN for the rhythmic control mechanism.

PMID: 8394182 [PubMed - indexed for MEDLINE]

1: J Am Soc Nephrol 1998 Dec;9(12):2223-34

# Effects of the angiotensin II antagonist valsartan on blood pressure, proteinuria, and renal hemodynamics in patients with chronic renal failure and hypertension.

Plum J, Bunten B, Nemeth R, Grabensee B.

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Angiotensin II receptor antagonists have become clinically available for the treatment of arterial hypertension. Presently, there is little information about their effects on BP, proteinuria, and renal function in patients with moderate or advanced renal failure. This study examines the effects of the angiotensin II antagonist Valsartan (80 mg/d) on proteinuria and glomerular permselectivity in patients with chronic renal failure during a 6-mo treatment, using a double-blind, randomized, placebo-controlled study (treatment group [V-group]: n = 5, age 57 +/- 7 yr, serum creatinine 365 +/- 122 micromol/L; placebo group [P-group]: n = 4, age 62 +/- 11 yr, serum creatinine 346 +/- 61 micromol/L). Study parameters included BP, 24-h proteinuria, GFR, and effective renal plasma flow (ERPF) as determined by inulin and para-aminohippurate clearance. Changes in glomerular permselectivity were assessed by measuring the fractional clearances of neutral dextrans by HPLC gel-permeation chromatography. Valsartan lowered the mean arterial pressure on average by 13 +/- 7 mmHg during the 6-mo treatment ($P < 0.05$). GFR and ERPF remained almost unchanged. However, after 6 mo of Valsartan treatment, proteinuria was reduced by 396 +/- 323 mg/24 h (26 +/- 18%) and albuminuria by 531 +/- 499 mg/24 h (41 +/- 21%) with regard to baseline values ($P < 0.05$). In the P-group, both proteinuria and albuminuria increased slightly with time (by 30 +/- 43% and 30 +/- 54%, respectively, NS). The fractional clearances of high molecular weight dextrans >66 A were significantly reduced after 6 mo of Valsartan treatment ($P < 0.05$), indicating a reduction of the glomerular shunt volume by 54 +/- 20% ($P < 0.05$) according to the model of Deen et al. (Am J Physiol 249: 347-389, 1985). The mean pore size radius of the glomerular membrane remained unchanged. This effect was independent of glomerular hemodynamic changes. Valsartan persistently lowered proteinuria in patients with chronic renal failure. Although GFR and ERPF remained nearly stable, this effect could be attributed to an improvement in glomerular permselectivity.

Publication Types:
Clinical trial
Randomized controlled trial

PMID: 9848776 [PubMed - indexed for MEDLINE]

1: Basic Res Cardiol 1998;93 Suppl 2:63-8.

# The angiotensin AT1 receptor antagonist CV-11974 regulates cerebral blood flow and brain angiotensin AT1 receptor expression.

Nishimura Y, Xu T, Johren O, Hauser W, Saavedra JM.

Section on Pharmacology, National Institute of Mental Health, Bethesda, MD 20892-1264, USA.

We studied cerebral blood flow autoregulation by laser Doppler flowmetry, and expression of brain angiotensin II AT1 receptors by quantitative autoradiography, after administration of an angiotensin AT1 receptor antagonist, CV-11974 (Candesartan, 0.5 or 1.0 mg/kg.day) for two weeks via subcutaneously implanted osmotic pumps in adult normotensive Wistar Kyoto and spontaneously hypertensive male rats (SHR). In SHR, the autoregulation curve was shifted towards higher blood pressures, when compared with that of normotensive Wistar Kyoto rats. Administration of CV-11974 shifted the autoregulation curve toward lower blood pressures in both Wistar Kyoto and SHR, partially normalizing the autoregulation curve in SHR. CV-11974 treatment markedly decreased the expression of AT1 receptors in Wistar Kyoto rats, both in areas outside the blood brain barrier (subfornical organ, 95% decrease) and inside the blood brain barrier (nucleus of the tractus solitarius, 87% decrease, and paraventricular nucleus, 96% decrease). Our results demonstrate that blockade of AT1 receptors tends to normalize the shift to higher pressures in the autoregulation curve of genetically hypertensive rats, and has a profound modulatory role in brain angiotensin II AT1 receptors.

PMID: 9833166 [PubMed - indexed for MEDLINE]

1: Behav Brain Res 1997 Sep;87(2):195-200

# Vasopressin and memory. I. The vasopressin analogue AVP4-9 enhances working memory as well as reference memory in the radial arm maze.

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The present study examined the effects of vasopressin on memory. Healthy rats were injected with the arginine vasopressin fragment AVP4-9 and the AVP antagonist [beta-mercapto-beta,beta-cyclopentamethylenepropionyl1,O-Et-Pyr 2,Val4,Arg8] and were tested in an eight-arm radial maze for 60 sessions. All injections were given s.c. 30 min prior to testing. AVP4-9 enhanced radial arm maze performance. AVP4-9 treated animals showed enhancement in performance as well as increases in the rate of learning, indicating that they learned the task faster. Furthermore, the overall memory enhancement was due to improved working memory as well as to improved reference memory. These results cannot be explained in terms of changes in locomotor activity because an open field test revealed no differences between groups for both of these compounds. The AVP antagonist did not impair performance in the radial maze. It is concluded that AVP4-9 has a more general effect on memory, one that is not limited to a specific type of memory.

PMID: 9331487 [PubMed - indexed for MEDLINE]

Appendix B

# Biotinylated Ligands

1: Blood 1995 Dec 1;86(11):4270-7

# Macrophage inflammatory protein-1 alpha receptors are present on cells enriched for CD34 expression from patients with chronic myeloid leukemia.

Chasty RC, Lucas GS, Owen-Lynch PJ, Pierce A, Whetton AD.

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The response of normal and chronic myeloid leukemia (CML), CD34+ cells to human macrophage inflammatory protein-1 alpha (MIP-1 alpha or LD78) was assessed. In tritiated thymidine incorporation assays, stem cell factor plus granulocyte-macrophage colony-stimulating factor stimulated thymidine incorporation in normal CD34+ cells was reduced to 72% of control values in the presence of MIP-1 alpha, whereas incorporation by CML CD34+ cells exposed to the same factors was not altered. In clonogenic assays, the presence of MIP-1 alpha gave a level of colony formation that was 71% of control values for normal progenitor cells, whereas for CML CD34+ cells colony formation was enhanced by 25%. These results suggest that, in vitro, CML progenitor cells are relatively refractory to the growth inhibitory effects of MIP-1 alpha. Using flow cytometry, the specific binding of a biotinylated human MIP-1 alpha/avidin fluorescein (FITC) conjugate to normal and CML mononuclear and CD34+ cell populations was quantified. The data indicate that (for both normal and CML CD34+ cells) there was a single population of cells that express cell surface receptors for MIP-1 alpha and this receptor expression was independent of cell cycle status. CML progenitor cells may be refractory to the effects of MIP-1 alpha as a result of events downstream from receptor expression.

PMID: 7492787 [PubMed - indexed for MEDLINE]

1: J Biol Chem 1997 Dec 12;272(50):31785-92

# Platelet factor 4 binds to glycanated forms of thrombomodulin and to protein C. A potential mechanism for enhancing generation of activated protein C.

Dudek AZ, Pennell CA, Decker TD, Young TA, Key NS, Slungaard A.

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Platelet factor 4 (PF4) is an abundant platelet alpha-granule heparin-binding protein. We have previously shown that PF4 accelerates up to 25-fold the proteolytic conversion of protein C to activated protein C by the thrombin.thrombomodulin complex by increasing its affinity for protein C 30-fold. This stimulatory effect requires presence of the gamma-carboxyglutamic acid (Gla) domain in protein C and is enhanced by the presence of a chondroitin sulfate glycosaminoglycan (GAG) domain on thrombomodulin. We hypothesized that cationic PF4 binds to both protein C and thrombomodulin through these anionic domains. Qualitative SDS-polyacrylamide gel electrophoresis analysis of avidin extracts of solutions containing biotinylated PF4 and candidate ligands shows that PF4 binds to GAG+ but not GAG- forms of thrombomodulin and native but not Gla-domainless protein C. Quantitative analysis using the surface plasmon resonance-based BIAcoreTM biosensor system confirms the extremely high affinity of PF4 for heparin (KD = 4 nM) and shows that PF4 binds to GAG+ thrombomodulin with a KD of 31 nM and to protein C with a KD of 0.37 &mgr;M. In contrast, PF4 had no measurable interaction with GAG- thrombomodulin or Gla-domainless protein C. Western blot analysis of normal human plasma extracted with biotinylated PF4 demonstrates PF4 binding to protein C in a physiologic context. Thus, PF4 binds with relative specificity and high affinity to the GAG- domain of thrombomodulin and the Gla domain of protein C. These interactions may enhance the affinity of the thrombin.thrombomodulin complex for protein C and thereby promote the generation of activated protein C.

PMID: 9388524 [PubMed - indexed for MEDLINE]

1: Peptides 1991 Mar-Apr;12(2):375-81

Biotinylation of a bombesin/gastrin-releasing peptide analogue for use as a receptor probe.

Anton PA, Reeve JR Jr, Rivier JE, Vidrich A, Schepp W, Shanahan F.

Department of Medicine, UCLA.

The development of a biotinylated bombesin/gastrin-releasing peptide (GRP) for use as a receptor probe is reported. The lysine13 of a GRP-27 was substituted by arginine and lysine was added to the amino terminus. Biotinylation of the N-terminal lysine was performed. The biotinylated peptide was purified by HPLC and characterized by mass spectral analysis. Binding studies with murine Swiss 3T3 fibroblasts, cells known to express bombesin/GRP receptors, yielded a dissociation curve for the biotinylated GRP-27 analogue (biotin-Lysyl[Asp12,Arg13]GRP-27) which was nearly identical to that of native GRP. Using studies of gastrin release from isolated canine G cells, equipotent functional activity of the biotinylated probe and unmodified GRP was demonstrated. Measurements of retained 125I-avidin confirmed that the biotin/avidin interaction could occur once the biotin-peptide complex was bound. Applicability of the probe was demonstrated with fluorescent microscopy using avidin-FITC on Swiss 3T3 fibroblasts. In conclusion, a novel biotinylated bombesin/GRP analogue has been developed which retains the functional characteristics of the native peptide and is a useful probe for receptor studies.

PMID: 1648717 [PubMed - indexed for MEDLINE]

1: Biochemistry 1990 Sep 25;29(38):8943-50.

# Affinity chromatography purification of angiotensin II receptor using photoactivable biotinylated probes.

Marie J, Seyer R, Lombard C, Desarnaud F, Aumelas A, Jard S, Bonnafous JC.

Centre CNRS-INSERM de Pharmacologie-Endocrinologie, Montpellier, France.

We have developed biotinylated photoactivable probes that are suitable for covalent labeling of angiotensin II (AII) receptors and the subsequent purification of covalent complexes through immobilized avidin or streptavidin. One of these probes, biotin-NH(CH2)2SS(CH2)2CO-[Ala1,Phe(4N3)8]AII, which contains a cleavable disulfide bridge in its spacer arm and which displays, in its radioiodinated form, very high affinity for AII receptors (Kd approximately 1 nM), proved to be suitable for indirect affinity chromatography of rat liver receptor with facilitated recovery from avidin gels by use of reducing agents. This constituted the central step of an efficient partial purification scheme involving hydroxylapatite chromatography, streptavidin chromatography, and thiopropyl-Sepharose chromatography. SDS-PAGE analysis and autoradiography established the identity of the purified entity (molecular weight 65K) as the AII receptor. Possible ways of completing purification to homogeneity and extrapolation of the protocols to a preparative scale are discussed, as well as the potential contribution of our new probes to the study of the structural properties of angiotensin receptors.

PMID: 2271569 [PubMed - indexed for MEDLINE]

1: J Biol Chem 1990 Aug 25;265(24):14599-605

# Biotinyl analogues of vasopressin as biologically active probes for vasopressin receptor expression in cultured cells.

Jans DA, Bergmann L, Peters R, Fahrenholz F.

Max-Planck-Institut fur Biophysik, Johann Wolfgang Goethe University, Frankfurt, Federal Republic of Germany.

Biotinyl analogues of [Arg8]vasopressin were synthesized with the biotinyl moiety at position 4. This involved the substitution of 2, 4-diaminobutyric acid (Dab) for Gln4 in [1-deamino-Arg8]vasopressin to give the parent peptide des-[Dab4,Arg8]vasopressin. Two biotinyl analogues with different spacers between the side chain of Dab4 and the biotinyl residue were then prepared and characterized in detail. The analogues retained high binding affinities for the V2-receptor in both bovine kidney membranes and LLC-PK1 renal epithelial cells and for the V1-receptor in rat liver membranes. Both analogues were as potent as [Arg8] vasopressin in stimulating the cAMP-dependent protein kinase and the production of urokinase-type plasminogen activator in LLC-PK1 cells, with concentration dependence consistent with receptor binding affinities. Avidin or streptavidin did not appear to reduce receptor binding or biological activity of the biotinyl analogues. The use of the biotinylated vasopressin analogue des-[Dab-(biotinylamido)hexanoyl4, Arg8]vasopressin together with fluorescein-labeled streptavidin as a fluorescent probe for the V2-receptor in LLC-PK1 cells demonstrated the following: 1) Specific binding of the biotinyl analogue shown by quantitative single-cell fluorescence measurements using the technique of fluorescence microphotolysis; 2) the V2-receptor visualized by fluorescence microscopy; and 3) the expression of the V2-receptor detected by flow cytometry.

PMID: 2143764 [PubMed - indexed for MEDLINE]

# Appendix B

## Anti-Biotin Antibodies

1: Methods Enzymol 1997;279:451-63

# Preparation and properties of anti-biotin antibodies.

Kohen F, Bagci H, Barnard G, Bayer EA, Gayer B, Schindler DG, Ainbinder E, Wilchek M.

Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel.

Publication Types:
Review
Review, tutorial

PMID: 9211297 [PubMed - indexed for MEDLINE]

1: J Immunol Methods 1993 Oct 15;165(2):177-82

# A comparison of the binding of biotin and biotinylated macromolecular ligands to an anti-biotin monoclonal antibody and to streptavidin.

Vincent P, Samuel D.

Laboratory of Microbiological Reagents, Central Public Health Laboratory, Colindale, UK.

A competitive enzyme immunoassay was used to study the binding of biotinylated macromolecular ligands and d-biotin to an anti-biotin monoclonal antibody and to streptavidin. Solid phase BSA-c-biotin competed with biotin or biotinylated macromolecular ligands in solution for receptor binding. The concentration of d-biotin required to inhibit streptavidin binding to solid phase BSA-c-biotin by 50% was 11.5 pM. This streptavidin-biotin interaction was taken as having an affinity/avidity index of 100 and all other receptor-ligand interactions were calculated relative to this. The avidity indices calculated for streptavidin interactions with BSA-c-biotin and IgG-biotin were 17.6 and 6.6 respectively, whereas for anti-biotin the values for these ligands were 20.5 and 19.9 respectively. The interaction of anti-biotin with d-biotin had an affinity index of 0.001. Although streptavidin has the greatest binding affinity for d-biotin, its avidity for biotinylated ligands was considerably lower and comparable to that observed for anti-biotin-biotinylated macromolecule interactions.

PMID: 8228268 [PubMed - indexed for MEDLINE]

1: FEBS Lett 1993 May 3;322(1):47-50

# Monoclonal anti-biotin antibodies simulate avidin in the recognition of biotin.

Bagci H, Kohen F, Kuscuoglu U, Bayer EA, Wilchek M.

Department of Hormone Research, Weizmann Institute of Science, Rehovot, Israel.

The sequence of the VH gene of a monoclonal anti-biotin antibody was determined. Biotin-binding motifs, similar to those in avidin and streptavidin, were identified in complementary determining regions 2 and 3, suggesting that natural selection of functional motifs may occur in unrelated protein types.

PMID: 8482366 [PubMed - indexed for MEDLINE]